

Chapter 2

Antitumor Effects of IL-12 in Preclinical Studies

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Abstract The strong ability of IL-12 to stimulate many different mechanisms of innate and adaptive immunity (see previous chapter) corresponded to very potent antitumor effects of this cytokine observed in early studies in animal models of tumor therapy. This cytokine has been found effective in dozens of transplantable murine tumors, involving carcinomas, sarcomas, melanoma, and also hematologic malignancies, including poorly immunogenic tumors. Since IL-12 often exerted unacceptable toxic effects on normal tissues, at least when injected systemically, many attempts have been made to improve its therapeutic index by combination of this cytokine with various therapeutic agents and modalities: antibodies, tumor vaccines, other cytokines, antiangiogenic agents, chemotherapy, radiotherapy, and adoptive immunotherapy (Golab and Zagozdzon in *Int J Mol Med* 3: 537–544, 1999; Lasek et al. in *Cancer Immunol Immunother* 63: 419–435, 2014). To further attenuate IL-12-induced toxicities, in many recent preclinical treatment schemes, gene therapy approaches have been tested in which the IL-12 gene is introduced in various viral and non-viral vectors. Some of these approaches have been and are being applied in clinical trials. This chapter provides an overview of the most promising IL-12-based experimental strategies that have been recently tested in animal models of neoplasia.

Keywords IL-12 · Animal models · Gene therapy · Chemotherapy · Immunotherapy

2.1 IL-12 in a Combination Treatment with Chemotherapy

Chemotherapy is a standard therapeutic modality in patients with many types of advanced cancer but it can potentially inhibit development of antitumor immunity interfering with proliferation and/or viability of cells of the immune system

responding against tumor. While passive immunotherapy, based on monoclonal antibodies, may improve the overall therapeutic effect of chemotherapy (e.g., trastuzumab and pembrolizumab in HER-2/neu-positive breast cancers), combination of chemotherapy with active immunotherapy—aimed at stimulation of the antitumor mechanisms—did not seem, due to anti-proliferative and immunosuppressive effects of many chemotherapeutics, a promising approach. However, there are strong rationales to combine chemotherapy with active immunotherapy, including IL-12, justifying continuation of investigations in animal models:

- chemotherapy may lead to complete clinical response but not necessary for the elimination of all tumor cells. Immunotherapy, due to high specificity, may completely eradicate the disease when used in adjuvant protocols, especially that some cytotoxic drugs render tumor cells more susceptible to killing by NK cells and cytotoxic T lymphocytes (Bergmann-Leitner and Abrams 2001; Wennerberg et al. 2013);
- certain anti-neoplastic agents at subtoxic doses can exert immunomodulatory effects by inhibition of Treg cells, reverting immunosuppressive mechanisms (Lutsiak et al. 2005; Schevchenko et al. 2013). Supplementation of immunostimulatory agents may further enhance antitumor response (Kersten et al. 2015; Nars and Kaneno 2013);
- chemotherapy, especially when used locally at the site of tumor development (for example, electrochemotherapy with cisplatin or bleomycin), induces releasing of tumor-associated antigens, exposure of calreticulin on apoptotic tumor cells, extracellular release of high-mobility group protein B1 (HMGB1) and ATP, defined as damage-associated molecular patterns (DAMPs). This so-called immunogenic cell death (Inoue and Tani 2014) may be compared to tumor vaccine deposition and may lead to improved presentation of tumor antigens by dendritic cells to T cells (Shurin et al. 2009). Subsequent application of active nonspecific immunotherapy, in particular IL-12, may facilitate or induce effective systemic antitumor response (Sersa et al. 2015).

Several chemotherapeutics, for example: cyclophosphamide, paclitaxel, doxorubicin, and cisplatin, have been shown to demonstrate augmented antitumor activity in mice and large animals (rabbits, dogs), when combined with IL-12 applied either in models of direct intratumoral or systemic injections of the cytokine or in gene therapy strategies.

Cyclophosphamide is an alkylating agent which has been the most frequently tested, among chemotherapeutics, in preclinical studies in combination with IL-12. While high doses of cyclophosphamide lead to immunosuppression due to reducing T cell proliferation, this agent—when used at subtoxic doses or in metronomic schedules—enhance specific immune response against antigens via inhibition of Treg cells and immunosuppressive mechanisms (Berd et al. 1982; Lutsiak et al. 2005; Ghiringhelli et al. 2007). In several animal tumor models, combination of IL-12 and cyclophosphamide exerted more potent antitumor effects than either agent alone. The mechanisms of the additive/synergistic effect of this combination are not

fully clear. Both selective depletion of Treg cells by cyclophosphamide and IL-12-mediated stimulation of NK and antitumor effector T cells, restored by cyclophosphamide, can be considered. IL-12 can act as an adjuvant for tumor antigens released from cyclophosphamide-killed cells and can induce Th1-dependent antitumor effect (Zhang 2013a). Further, treatment with cyclophosphamide and IL-12 induces NKT cells (Karnbach et al. 2001) and depletes myeloid-derived suppressor cells (MDSC) in the tumor tissue and in the spleen (Malvicini et al. 2012; Medina-Echeverez et al. 2011; Rossowska et al. 2014). However, some authors emphasize that preexisting antitumor immunity is the critical factor for improved antitumor effects of cyclophosphamide (and also some other chemotherapeutics) + IL-12 combination (Le et al. 2001; Zhang et al. 2013a). Human cancers are poorly immunogenic at advanced, metastatic stages. Nonetheless, the immunoenhancing properties of cyclophosphamide were the rationale to combine this agent with antitumor vaccines in some clinical trials (Walter et al. 2012) (see also next chapter, NCT02498912).

Apart from cyclophosphamide, a number of other chemotherapeutic drugs have been successfully tested in combination with IL-12 in preclinical models against various tumors. In contrast to studies from the turn of last and current century, in which IL-12 was mainly used as a recombinant protein in the combination treatment (Golab et al. 2001; Teicher et al. 1997; Zagodzón et al. 1998, 1999), current experimental chemo-immunotherapy approaches in animal tumor models preferentially combine chemotherapeutics with IL-12 gene therapy. An interesting idea of joint therapy is the combination of local electrochemotherapy with IL-12 gene tissue transfer via electroporation (Sersa et al. 2015). This treatment imitates *in situ* vaccination: high concentration of tumor antigens are released from drug-destroyed tumor cells in the context of IL-12, acting as a biological adjuvant. Independently, systemic side effects of chemotherapy are greatly reduced. Encouraging results were obtained, for example, in models of murine sarcoma and carcinoma in mice treated with cisplatin-based electrochemotherapy and *i.m.* IL-12 plasmid (pIL-12) electrotransfer (Sedlar et al. 2012). The combined electrochemotherapy and pIL-12-based electroimmunotherapy was also found effective in spontaneous tumors in dogs in the treatment in which bleomycin, cisplatin, or gemcitabine were administered with pIL-12 *i.t./peritumorally* (Cutrera et al. 2015; Cemazar et al. 2016). Another example of a novel experimental IL-12-based therapy that seems to have clinical potential is the combination of IL-12-secreting oncolytic herpes simplex virus (HSV)-1 with chemotherapy. Encouraging results were demonstrated in the combined treatment consisting of the HSV-1 vector engineered to produce IL-12 with *i.p.* vinblastin in a prostate cancer model in mice (Passer et al. 2013). Of note, the clinical trial has been commenced with genetically engineered oncolytic HSV-1 expressing IL-12 gene to treat patients with recurrent/progressive glioblastoma multiforme, anaplastic astrocytoma, or gliosarcoma (NCT02062827). The most promising current variants of IL-12-based antitumor chemo-immunotherapy in animal models are presented in Table 2.1.

Table 2.1 Antitumor effects of interleukin 12 potentiated by various chemotherapeutic modalities in current experimental models

Chemotherapeutic/toxic agent	IL-12	Animals	Tumor/tumor cell line	Comments	References
Bleomycin i.t. (+electrop.) or i.v.	pIL-12 i.t. (+electrop.) or peritum.	Dogs	Spontaneous tumors		Cutrer et al. (2015) Cenazar et al. (2016)
Bortezomib, i.v.	rIL-12, i.p.	SCID mice	RPMI8226 human melanoma		Wang et al. (2014)
Cisplatin i.t. (+electrop.) or i.v.	pIL-12 peritum. (+electrop.)	Dogs	Spontaneous tumors		Cenazar et al. (2016)
Cisplatin i.t. (+electrop.)	pIL-12 i.m. (+electrop.)	Mice	B16 melanoma		Kim and Sin (2012)
Cisplatin i.t. (+electrop.)	pIL-12 i.m. (+electrop.)	Mice	SA-1 sarcoma, TS/A carcinoma		Sedlar et al. (2012)
Cyclophosphamide, i.p.	rIL-12, i.p.	Mice	MCA207 sarcoma, C26 colon cancer		Zhang et al. (2015a)
Cyclophosphamide, i.p. (low dose, single dose or metronomic schedule)	AdIL-12, i.t.	Mice	CT26 colorectal carcinoma	Single dose is superior to metronomic schedule	Malvicini et al. (2012)
Cyclophosphamide, i.p.	rIL-12, i.p.	Mice	MCA207, MCA203, Panc02		Zhang et al. (2013a)
Cyclophosphamide, p.o. (metronomic schedule)	pIL-12 i.t. (+electrop.)	Mice	B16-F10 melanoma	Combination with human tyrosinase (hTyr) pDNA	Denies et al. (2014)
Cyclophosphamide, i.p.	pIL-12 hydrodynamic	Mice	MC38 colon carcinoma		Medina-Echeverz et al. (2011)
Cyclophosphamide, i.p.	DC-IL-12 peritum.	Mice	MC38 colon carcinoma		Rossowska et al. (2014)
Docetaxel, i.p.	NHS-muIL12*, s.c.	Mice	MC38 colon carcinoma		Fallon et al. (2014)

(continued)

Table 2.1 (continued)

Chemotherapeutic/toxic agent	IL-12	Animals	Tumor/tumor cell line	Comments	References
D-K ₆ L ₉ peptide, i.t.	pIL-12, i.t.	Mice	B16-F10 melanoma, C26 colon carcinoma		Cichon et al. (2014)
Doxorubicin, i.p.	pIL-12 i.m. (+electrop.)	Mice	4T1 breast carcinoma		Hu et al. (2014)
Doxorubicin (liposomal), i.t.	rIL-12, i.p.	Mice	MCA207, MCA203, Panc02		Zhang et al. (2013a)
5-Fluorouracil, i.p.	rIL-12, i.p.	Mice	L1210 leukemia		Golab et al. (2001)
Gemcitabine i.t. (+electrop.)	pIL-12 i.t. (+electrop.)	Dogs	Spontaneous tumors		Cuttrera et al. (2015)
Gemcitabine, i.t.	rIL-12, i.p.	Mice	MCA207, MCA203, Panc02		Zhang et al. (2013a)
Gemcitabine, i.v.	NHS-muIL12* s.c.	Mice	Panc02 pancreatic tumor cells		Fallon et al. (2014)
Mitomycin, i.a.	pIL-12, i.a.	Rabbits	Hepatocellular carcinoma		Xia et al. (2013)
Paclitaxel, i.t.	AdIL-12, i.t.	Mice	B16 melanoma	Therapeutics incorporated in anionic liposomes	Cao et al. (2013)
Sorafenib, p.o.	DC-IL-12, i.t.	Mice	Hepatocellular carcinoma	DC-IL-12 - tumor lysate pulsed DC	Vogt et al. (2014)
Sunitinib, p.o.	NHS-muIL12*, s.c.	Mice	Renca kidney tumor cells		Fallon et al. (2014)
Vinblastin, i.p.	oHSV-IL-12, i.t.	Nude mice	CWR22 human prostate cancer		Passer et al. (2013)

Abbreviations i.a. intraarterially, i.m. intramuscularly, i.p. intraperitoneally, i.t. intratumorally, i.v. intravenously, peritum. peritumorally, p.o. per os, s.c. subcutaneously, electrop. electroporation, pIL-12 IL-12 plasmid, rIL-12 recombinant IL-12

*NHS-muIL12—immunocytokine targeting DNA at tumor necrosis sites

2.2 Combination of IL-12 and Radiotherapy

Radiotherapy is commonly used to treat various types of cancer, either alone, as an adjuvant therapy, or in combination settings. Some of the most successful combinations of radio- and immunotherapy in animal models were the base to initiate clinical trials (for review see Formenti and Demaria 2013). Radiation-induced immunogenic cell death and enhanced expression of death receptors and MHC class I molecules on tumor cells (Golden and Apetoh 2015) was the rationale for combining IL-12 with radiotherapy in early experiments in animal models (Seetharam et al. 1999; Xian et al. 2005). Combining radiotherapy and IL-12 may be additionally beneficial since IL-12 could not only potentiate antitumor effects of radiotherapy but could diminish acute radiotherapy injury, according to observations in mice (Gerber et al. 2015) and in nonhuman primates (Basile et al. 2012). The advantage of IL-12 combination with irradiation has been recently reinforced by the observation of general inhibitory effect of radiotherapy on IL-12 production in mice (Lee et al. 2016).

Due to systemic toxicity of IL-12, most recent preclinical studies are aimed at combination of radiotherapy with IL-12 gene therapy. Encouraging results were obtained, for example, in a combination intratumoral treatment with oncolytic adenovirus expressing IL-12 and GM-CSF with radiation in a murine hepatic cancer model (Kim et al. 2011). Augmentation of the antitumor effects was also demonstrated by combining electrotransfer of the plasmid coding for IL-12 (i.t.) with local tumor irradiation in a model of sarcoma in mice (Sedlar et al. 2013). In studies by Jeong et al. (2015), bone marrow-derived mesenchymal stem cells were successfully used as IL-12 gene-delivery vehicles in conjunction with irradiation. The combined treatment led to suppression of pulmonary metastasis and improved survival in a metastatic hepatoma model in mice. Very interesting combinatory approach, called radiation-induced transcription targeting, was proposed by Kamensek et al. (2013). They used combined radio-gene therapy with the plasmid encoding IL-12 under the control of the radiation-inducible p21 promoter in a mammary adenocarcinoma model in mice. Although the overall therapeutic effect of the treatment was comparable to that using the constitutive promoter, serum and intratumoral concentrations of IL-12 were significantly lower in the former variant of therapy (Kamensek et al. 2013). One may consequently expect less adverse events after radio-gene therapy with inducible p21 promoter controlling IL-12 production.

2.3 IL-12-Based Combination Treatment with Immunotherapy

Current trends in IL-12-based combination immunotherapy can be generally divided into that aimed at potentiation of specific mechanisms of the antitumor response (e.g., in cancer vaccine approach or adoptive T cell therapy) and those in which

IL-12 is applied as a supplement to other immunotherapeutics, biotherapeutics, and so on, differed in antitumor mechanisms to that of IL-12.

When used as a cancer vaccine adjuvant, IL-12 was found more or less effective in many early preclinical models of specific antitumor therapy, namely in cancer vaccination approach (Portielje et al. 2003). In most cases, a prerequisite of the therapeutic effect of the cytokine was induction of cell-mediated immunity. The list of protocols and most promising variants of the combined therapy, in which IL-12 has been applied preferentially as gene therapy, include:

- coadministration of IL-12 with tumor cells (killed or specifically prepared), tumor cell elements, and tumor peptides (either native or modified) (Chang et al. 2016; Yin et al. 2015; Miguel et al. 2013; Jarosz-Biej et al. 2015);
- application of cancer cells transduced/transfected with IL-12 gene (Switaj et al. 2004; Thaci et al. 2014);
- cancer vaccine therapy based on dendritic cells or monocytes, either engineered to express IL-12 or co-injected with IL-12 (Zapala et al. 2013; Huang et al. 2012; Tan et al. 2013; Wang et al. 2016).

Some studies in animal models have been directed to augment the antitumor effects of IL-12 by combination of this cytokine or IL-12 gene therapy with antiangiogenic agents, for example with cilengitide (EMD121974) (Martin et al. 2014), ABRaA-VEGF₁₂₁ (Ciomber et al. 2014), vasostatin (Yao et al. 2000), angiostatin, endostatin, or saxatilin (Kim et al. 2013b; Zhang et al. 2013b). This approach seems to be especially effective in the prevention or inhibition of the metastatic process (Martin et al. 2014).

Other strategy to potentiate the effect of IL-12 is its combination with immunostimulatory cytokines. The most beneficial approach seemed to join IL-12 with IL-2 but clinical trials ended unsuccessfully (Weiss et al. 2007). Improved variant of this therapy, based on Newcastle disease virus strains expressing both IL-2 and IL-12, has been recently tested in a hepatoma model in mice (Ren et al. 2015). The panel of other cytokines that were tested in combination with IL-12 in preclinical studies includes also: IL-15, IL-18, IL-27, GM-CSF, TNF- α , and IFN- α (Lasek et al. 2004; Lesinski et al. 2004; Weiss et al. 2007; Basak et al. 2008; Sabel et al. 2010; Zhu et al. 2010; Choi et al. 2011; Kim et al. 2011; Ardolino et al. 2014). The combination of IL-12 and IL-15 seems especially promising since IL-12 induces upregulation of IL-15R α , resulting in enhancement of both innate and adaptive antitumor immunity (Lasek et al. 1999; Quetglas et al. 2013). The benefit of combining IL-12 and IL-18, on the other hand, results from reversing of NK cell anergy—at least in hosts bearing MHC class I-deficient tumors (Ardolino et al. 2014).

Several research groups tested combination of IL-12 with monoclonal antibodies in animal models. The Ohio State University group (Columbus, OH, U.S.A) showed encouraging results of joint therapy with IL-12+ anti-HER2/neu mAb (an analogue of human trastuzumab) in a mice model of colon adenocarcinoma expressing human HER2/neu antigen (Jaime-Ramirez et al. 2011). The group tested the same combination (IL-12 with trastuzumab, and also with paclitaxel) in a phase

I clinical trial recruiting patients with HER2-overexpressing cancers (breast, colon, stomach, and other cancers) (Bekaii-Saab et al. 2009). The combination therapy exhibited an acceptable toxicity profile and patients who experienced clinical benefit in response to the treatment showed higher levels of IFN- γ and IL-12-induced chemokines in blood when compared with nonresponders. Of note, clinical responses were only observed following addition of IL-12 to the treatment regimens in the second cycle of chemotherapy + trasuzumab and all but one response occurred in patients with the highest expression of HER2 in the tumor (immunohistochemistry 3+ samples). The same research group tested also IL-12 + cetuximab mAb (anti-EGFR) combination in athymic mice xenografted with human head and neck cancer cells (Luedke et al. 2012). Potentiated therapeutic effects of IL-12 used in combination with antitumor antibodies are probably caused by increased expression of activating Fc γ receptors on effector cells and augmented activity of NK cells participating in antibody-dependent cell-mediated cytotoxicity (Parihar et al. 2002; Campbell et al. 2015).

Recently, promising results were obtained in animal studies of the combined application of IL-12-based therapy with mAbs blocking molecules involved in suppression of the antitumor response (so called immune-checkpoint inhibitors): anti-CTLA-4 (vom Berg et al. 2013), and anti-PD-L1 (Bozeman et al. 2016). In the latter study, IL-12 was a glycolipid-anchored (GPI) element of cellular vaccines expressing also immune costimulatory B7-1 (CD80) molecule. Augmentation of the antitumor effect of IL-12 (precisely, i.t. injections of Semliki Forest virus encoding IL-12) was also obtained in the combination treatment with agonistic (immune stimulatory) anti-CD137 mAb in melanoma and lung carcinoma models in mice (Quetglas et al. 2012). Worth noting, both blocking and stimulatory mAbs against surface molecules are intensively studied in current tumor immunotherapy strategies in clinical oncology (Buque et al. 2015). Preliminary clinical data were presented very recently at AACR Annual Meeting 2016, demonstrating the effect of combination treatment with anti-PD-1 mAbs (pembrolizumab) and electroporation of i.t. IL-12 plasmid in a group of patients with melanoma (Algazi et al. 2016).

Very promising and actively explored IL-12-based approach in preclinical studies (and also in current clinical trials) is improving adoptive T cell therapy by transduction of effector cells with IL-12 gene. There have been several variants of this approach in recent investigations:

- application of TCR-engineered T cells, targeting tumor-specific antigen, expressing independently IL-12 (Zhang et al. 2011; Kerkar et al. 2013; Galvan et al. 2015);
- treatment with IL-12-secreting T cells expressing tumor-targeted chimeric antigen receptors (CAR) (Chmielewski et al. 2011; Pegram et al. 2012; Koneru et al. 2015; Pegram et al. 2015);
- treatment with IL-12-engineered NK cells (Zhu et al. 2012).

Both TCR- and CAR-engineered cells modified to produce IL-12 have the advantage to deliver IL-12 in a controlled fashion preferentially to the tumor tissue

but, as findings of preliminary studies with cancer patients show, application of the cells does not necessary translate into the clinical success (Zhang et al. 2015b). Tremendous heterogeneity in human cancer cell phenotypes, short life span of infused CAR T cells, accidental accumulation, and toxic effects on normal tissues and organs may hamper feasibility of the CAR strategy in humans (Chmielewski and Abken 2015; Yeku and Brentjens 2016).

2.4 Improved IL-12 Delivery Methods to Increase the Cytokine Expression in the Tumor or to Prevent and Control Side Effects Related to the Treatment with IL-12

Early studies with IL-12 in preclinical models demonstrated that the amount of this cytokine at the tumor site is critical for tumor eradication (Colombo et al. 1996). It soon became evident that IL-12 cannot be administered systemically at potentially therapeutic doses in clinical oncology due to high toxicity. During the last decade, the role of immunosuppressive factors in the tumor environment has been strongly suggested as a cause of failure of many immunotherapeutic approaches and local treatment with immunostimulatory agents for cancer immunotherapy, as an alternative for systemic administration (if possible), has been recommended (Fransen et al. 2013). In line with this idea, novel therapeutic modalities, using IL-12, are an area of intensive exploration. Recent trends are mostly aimed at development of formulations providing sustained, high concentration of IL-12 in the tumor microenvironment while minimizing systemic dissemination of the cytokine. They involve, among others, studies on nanocarrier-based IL-12 delivery systems (liposomes, polymeric nanoparticles, or nanogels), application of bacterial (*Clostridium sporogenes*, *Lactococcus lactis*) or improved viral vectors (Semliki Forest virus, oncolytic HSV-1) and even infusions of mesenchymal stem cells expressing IL-12. In case of the latter approach, the advantage is tumor-homing capacity of mesenchymal stem cells. The above-mentioned experimental therapeutic strategies are summarized in Table 2.2. Worth noting is that some variants of local therapy with IL-12 delivery systems have been an area of active exploration in clinical trials. These include, for example, studies on nanoparticles containing human IL-12 plasmid (pIL-12/PPC, GEN-1) or i.t. application of pIL-12 + electroporation, and are described in the next chapter.

A novel strategy to regulate local production of IL-12 is the application of viral vector constructs containing IL-12 gene under the control of heat shock protein 70B gene promoter. Feasibility of this hyperthermia-induced gene therapy was demonstrated in Hep3B human hepatoma model in nude mice (Wei et al. 2013) and spontaneous feline soft tissue sarcomas (Siddiqui et al. 2007). Another delivery method to confine IL-12 expression to the tumor was reported by Lorenzo et al. (2015). They invented inflammation-regulated expression system based on

Table 2.2 Current trends in controlled delivery of IL-12 in preclinical models of neoplasia

IL-12 carrier system/vector	Additional treatment (compound)	Animals	Tumor model	Comments	References
<i>Nanoparticle carriers</i>					
Cationic liposomes containing pIL-12 (pVITRO2-FUS1-hIL-12), i.v.	FUS1 suppressor gene plasmid	SCID mice	Lung metastatic disease		Ren et al. (2014)
Cationic liposomes containing IL-12, i.t.	Monophosphoryl lipid A (MPL)	Mice	4T1 breast carcinoma		Meraz et al. (2014)
Cationic liposome/IL-12 pDNA complex, i.v.	All- <i>trans</i> -retinoic acid	Mice	Colon26 adenocarcinoma, metastatic model		Charoensit et al. (2010)
IL-12 incorporated in CHP (cholesterol-bearing pustullan) nanogel (CHP/rmIL-12), s.c.		Mice	CSA1M fibrosarcoma		Shimizu et al. (2008)
Liposomes complexed with pIL-12, i.p.		Mice	CT26 colon cancer	Folate receptor α -targeted liposomes	Luo et al. (2015)
Biopolymer chitosan with IL-12 (CS/IL-12), intravesically		Mice	Transitional cell carcinoma MB49 and MBT-2	Superiority of CS/IL-12 to BCG was shown in earlier studies	Smith et al. (2015)
Biopolymer chitosan with IL-12 (CS/IL-12), i.t.		Mice	4T1 breast carcinoma		Vo et al. (2014)
Poly-lactic acid microspheres (PLAM) containing IL-12, i.t.	IL-18, TNF- α	Mice	4T1 breast carcinoma		Sabel et al. (2010)
Cationic polyphosphazene vesicles (polymersomes) containing pIL-12, i.v.		Mice	CT-26 colon cancer		Gao et al. (2016)
(continued)					

Table 2.2 (continued)

IL-12 carrier system/vector	Additional treatment (compound)	Animals	Tumor model	Comments	References
IL-12 pDNA (+electrop.) i.t.		Dogs	Different spontaneous tumors		Cutrer et al. (2014)
IL-12 pDNA (scIL-12/CMVpDNA), i.m.		Mice	<i>Braf^{V600E}</i> -induced papillary thyroid carcinoma	Prophylactic administration of IL-12 pDNA led to prolonged mice survival	Parhar et al. (2016)
<i>Viral/bacterial vectors</i>					
<i>Clostridium sporogenes</i> expressing IL-12, i.v.		Mice	EMT6 mammary carcinoma, i.v.	Colonization in hypoxic tumor tissue but not in normal tissues	Zhang et al. (2014)
<i>Lactococcus lactis</i> expressing IL-12 (LL-E7-IL-12 _D), intranasal	E7 gene of HPV-16	Mice	E7-expressing TC-1 lung tumor		Li et al. (2014)
Oncolytic herpes simplex virus (HSV-1) expressing IL-12 (M002), i.t.		Mice	SCK mammary carcinoma, intracranial metastasis model	Clinical trial has been started with M032 oncolytic HSV-1 (NCT02062827)	Cody et al. (2012)
Oncolytic herpes simplex virus (oHSV) expressing IL-12 (G47Δ-mIL12), i.t.	oHSV expressing angiostatin	Mice	U87 intracranial glioma	Clinical trial has been started with M032 oncolytic HSV-1 (NCT02062827)	Zhang et al. (2013b)
Oncolytic adenovirus encoding IL-12 gene (AdΔB/IL-12), i.h.	AdΔB/TRAIL	Nude mice	Hep3B hepatocellular carcinoma		El-Shemi et al. (2016)
Oncolytic adenovirus expressing IL-12, i.t.	IL-18 co-expression	Mice	B16-F10 melanoma		Choi et al. (2011)
Semliki Forest virus vector expressing IL-12 (SFV-IL-12), i.t.	Attenuated strain of <i>Salmonella Typhimurium</i> (LVRO1) i.t.	Mice	4T1 breast carcinoma		Kramer et al. (2015)

(continued)

Table 2.2 (continued)

IL-12 carrier system/vector	Additional treatment (compound)	Animals	Tumor model	Comments	References
Semliki Forest virus vector expressing IL-12 (SFV-IL12), i.t.		Mice	MC38 colon carcinoma, TC-1 epithelial lung-transformed cells	Vector-induced type 1 IFNs strongly contribute to antitumor efficacy of IL-12	Mehero et al. (2015)
Sendai virus-based virosomes (cationic F/HN virosomes*) containing IL-12 pDNA, i.v.	Salmosin gene, doxorubicin	Mice	SKOV-3 ovarian cancer	Anti-EGFR antibody was coupled to the surface of virosomes	Kim et al. (2016)
<i>IL-12-expressing cells</i>					
Mesenchymal stem cells expressing IL-12 (Lenti-mIL-12-MSC), i.p.		Mice	MethA fibrosarcoma injected i.p.	MSCs were prepared from bone marrow	Han et al. (2014) Wang et al. (2015)
Mesenchymal stem cells expressing IL-12 (MSCs-IL-12M), i.t., i.v. or s.c.		Mice	B16F10 melanoma, TC-1 cancer cells	I.t. injection of MSCs-IL-12M is more effective than s.c. way; MSCs were prepared from bone marrow	Seo et al. (2011)
Mesenchymal stem cells expressing IL-12 (MSCs-IL-12), i.t.	Irradiation	Mice	HCa-1 and Hepa 1-6 hepatomas	MSCs were prepared from bone marrow	Jeong et al. (2015)

Abbreviations i.h. intrahepatically, i.m. intramuscularly, i.p. intraperitoneally, i.t. intratumorally, i.v. intravenously, s.c. subcutaneously, electroporation, EGFR epidermal growth factor receptor, MSC mesenchymal stem cell

*The system consists of cationic O₃O₃-dimiristyl-N-lysyl glutamate, Sendai virus fusion protein and hemagglutinin-neuraminidase protein

self-processing polypeptide muIL12opt-P2A. In this system, a local IL-12 release is controlled due to the presence of inflammation-induced promoters in the lentiviral vector containing IL-12 gene. Inducibility of the system was confirmed in vivo using murine LLC cells. An interesting way to enhance IL-12 delivery for brain tumor immunotherapy in a C6 glioma model in rat was proposed by Chen et al. (2015). They used focused ultrasound energy (transcranially) combined with i.v. microbubble administration to disrupt temporarily blood–brain barrier (BBB). The procedure enabled glioma tissue penetration of systemically administered IL-12. Importantly, increased permeability of BBB for IL-12 was associated with increased accumulation of effector T cells in the tumor, translating into improved glioma treatment efficacy.

An alternative way of tumor-targeted delivery of IL-12 is antibody-directed transfer of IL-12. Several Ab-IL-12 experimental biotherapeutics (immunocytokines) have been tested in experimental animal models and also in large animals (dogs). The agents consisted of one or two molecules of IL-12 conjugated with full antibodies (Paoloni et al. 2015; Fallon et al. 2014) or with smaller scFv fragments (Jahn et al. 2012; Kim et al. 2013a). In some studies, effectiveness of IL-12 immunocytokines has been augmented by co-delivery/complexing with other cytokines: IL-2 (Jahn et al. 2012; Schilbach et al. 2015), IL-4 (Hemmerle and Neri 2014), IL-7 (Schilbach et al. 2015), or when combined with radio- or chemotherapy (Fallon et al. 2014; Eckert et al. 2016). Worth noting is that the IL-12 immunocytokine-based antitumor therapy entered the stage of phase 1 clinical trial (Rudman et al. 2011, NCT01417546).

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